Characterization of *E. coli* Strains Obtained from Wastewater Effluent

Grażyna A. PŁAZA[1*], Agnieszka TUREK[1], Renata SZCZYGŁOWSKA[2]

[1] Institute for Ecology of Industrial Areas, Department of Environmental Microbiology, 40-844 Katowice, Poland

[2] "Strzegowa" Water Company, Research Accredited Laboratory, 63-500 Ostrzeszów, Piastowska 105, Poland

pla@ietu.katowice.pl

Abstract

The aim of the study was the characterization of *E. coli* isolates obtained from wastewater effluent. The following features were evaluated: enzymatic profile by API ZYM test, antibiotic resistance, biocide susceptibility, and hydrophobicity by BAH and SAT methods. The similar enzymatic pattern was obtained for all number of *E. coli*. Among the tested isolates O157:H7 *E. coli* were no identified. *E. coli* isolates were tested regarding their resistance to 20 different antibiotics. 90% of *E. coli* isolated were categorized as resistant. The highest resistance frequencies were found for the following antibiotics: cephalotin and erythromycin (90%), nitrofuzantoin (94%), rifampicin (97%) and novobiocin (100%). The collection of bacterial strains was also analyzed for multiple antibiotic resistance (MAR). The data indicated that 88% of bacteria showed resistance to 8 - 15 antibiotics. 3 % of isolates were resistance to 19 tested antibiotics. No strain was detected to be resistance from 0 to 5 antibiotics. The decrease of susceptibility of bacterial isolates to antibiotics was probably caused by the presence of these compounds in the wastewaters and the long exposition of *E. coli* strains to them. The isolates were susceptibility to tested biocides. Among the Preventol® biocides, the D2 type was the most active. The similar activity showed LV508 type from the Acticide® biocides. Only 17% of the isolates revealed moderate hydrophobicity.

Keywords

E. Coli Strains; Antibiotics; Biocide;, Hydrophobicity

Introduction

Lotin and enterococci are taken as the indicators of fecal contamination. Those bacteria are present in the intestinal tract of humans; however, they have been also frequently related to the human and animal diseases. However, wild *E. coli* are robust and potentially aggressive microorganisms that have evolved the ability to competitively colonise the intestines of mammalian hosts, survive free in the environment and sometimes cause clinical disease. *Escherichia coli* is present as normal flora in the lower intestine of both humans and animals; while some strains can cause gastrointestinal illnesses ranging from mild diarrhea to cholera-like diarrhea to potentially fatal complications, such as hemolytic uremic syndrome (HUS) (Coia 1998). Other Shiga-toxin producing *E. coli* (STEC) including the O157:H7 serotype cause hemorrhagic colitis and HUS (Coia 1998).

Lotin as fecal contamination may be transmitted to humans if these foods are improperly cooked or otherwise mishandled. The level of antibiotic resistance in *E. coli* represents a useful indicator of the resistance dissemination in bacterial populations. There are some reports in which antibiotic susceptibility of *E. coli* isolates from healthy humans or animals have been studied (Saenz et al. 2001; Sunde et al. 1998; Al-Ghamdi et al. 1999). Other important sources of *E. coli* as fecal contamination are sewage sludges and wastewater effluents used in the agriculture which is often associated with significant health risks (Muela et al. 2011). Taking into account the scarcity of conventional water sources, population growth and the agricultural water usage, there is need to use alternative water sources for agriculture with high-quality water required for human consumption (Palese et al. 2009). The reuse of wastewater for irrigation is one way to reduce water shortage. The wastewaters used for irrigation could be associated with minimal health and environmental risk, then microbiological quality standards for the purposes should be established (Palese et al. 2009).

The objective of the study was to characterize Lotin strains recovered from wastewater effluent.

Materials and Methods

Description of the Wastewater Treatment Plant and Sample Collection

Strzegowa wastewater treatment plant (WWTP) is situated close to Ostrzeszow urban area and located

on 20 h area of the Wielkopolska region (west part of Poland). The Company operates in the city and district Ostrzeszów and Mikstat, and it is responsible for municipal industrial, rain and wastewater treatment, technical maintenance of the sewage pumping stations and study quality of water and waste in own accredited laboratory.

Design capacity of the plant is between 7,500 and 12,000 m^3/d of wastes, with medium 5,000 m^3/day.

The treatment processes (40,000 equivalent population) used by the plant include a conventional activated sludge secondary treatment process. Sludge is treated in A2/O (modified A/O with anaerobic, annoxic (denitrification) and aerobic cells in sequence. In the process of purification, a high degree of pollution reduction is achieved(97% BOD, 93% COD, 94% total suspension, 83% total phosphorus, 77% total nitrogen).

50 wastewater samples (500 ml) collected during the spring and summer of 2012 were taken from treated wastewater effluents after the activated sludge processand stored in polypropylene bottles at 4◦C for microbiological analysis before 24 h of sampling.

Isolation and Identification of E. Coli and E. Coli O157:H7

Bacteria *E. coli* were isolated from the effluents and identified according to EN ISO 9308-1:2000 [7]. The RapidChek *E. coli* lateral Flow Test Kit was applied to detect *E. coli* O157 (including H7). The identification was performed according to the user guide. This immunoassay test uses a double antibody sandwich format. An antibody specific for *E. coli* O157 is sprayed and immobilized in a line on the surface of a membrane comprising a "test line". A second antibody reagent, also recognizing *E. coli* O157 and labeled with colloidal gold, is contained within a reagent pad upstream from the test line. As the sample moves by capillary action from the filter pad, the antibody gold reagent specifically binds *E. coli* O157 and moves with the liquid sample into the test membrane. The sample passes through the test line where the immobilized second *E. coli* antibody captures the protein-antibody-gold complex, causing the formation of an antibody-protein "sandwich" and development of red color at the test line. Antibody-protein sandwiches are not formed in the absence of *E. coli*, resulting in no red color development at the test line. Reagents immobilized at the control line capture excess gold reagent passing through the test line. The presence of red color at the control line indicates that the test strip flowed smoothly. Therefore, the presence of only one line (control line) on the membrane indicates a negative sample and the presence of two lines indicates a positive sample.

Biochemical Analysis of Isolated E. Coli

The biochemical characterization was based on the API ZYM test (bioMerieux S.A.). The test was performed by the producer instruction. API ZYM, a semi-quantitative micromethod designed for the research of enzymatic activities, allows the rapid study of 19 enzymatic reactions using very small sample quantities. The system consists of a strip with 20 microwells, containing synthetic substrates and their buffer.

The enzymatic tests were inoculated with 65 µl of bacterial suspensions with a turbidity of 5-6 McFarland. The inoculated strips were incubated 4 hours at 37◦C. After incubation, 1 drop of ZYM A and 1 drop of ZYM B reagents were added to each cupule. The specific color of the enzymatic reactions was developed for at least 5 minutes. The results were read according to the result sheet.

The following enzymes were analysed: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chmotrypsin, acid phosphatase, naphthol-AS-Bi-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase.

Isolates were maintained on SMA slants (8 g peptone, 2.5 g yeast extract, 1 g glucose and 20 g agar per litre, bioMerieux) at 4◦C for the analysis.

Evaluation of Antibiotics and Biocides Resistance

Antibiotic susceptibility tests of the isolates were carried out against 20 different antibiotics by the standard agar disk diffusion method. The strains were grown in SMA broth at 37◦C for 24 h. Bacterial suspensions were adjusted to OD_{600nm} 0.5 (ca. 10^7-10^8 CFU x dm^{-3}). Then, the incubated suspensions (0.1 mL) were spread onto Mueller-Hinton agar plates (Oxoid). The antibiotic-impregnated disks (Oxoid) were put on these freshly prepared lawns and incubated at 37◦C for 24 h and 48 h. The inhibition zone diameter was measured, and isolates were classified as resistant (R),

intermediate (I) and susceptible (S). The antibiotics used in this test were as follows (their concentration given in parentheses): amoxicillin (AML, 25 μg), ampicillin (AMP, 25 μg), ceftazidime (CAZ, 30 μg), cephalothin (KF, 30 μg), cefuroxime (CXM, 30 μg), nalidic acid (NA, 30 μg), amikacin (AK, 30 μg), doxycycline (DO, 30 μg), erythromycin (E, 30 μg), gentamycin (GN, 30 μg), kanamycin (K, 30 μg), neomycin (N, 30 μg), streptomycin (S, 25 μg), tobramycin (TOB, 10 μg), tetracycline (TE, 30 μg), trimethoprim (W, 5 μg), rifampicin (RD, 30 μg), chloramphenicol (C, 30 μg), nitrofurantoin (F, 20 μg), novobiocin (NV, 30 μg).

All tested antibiotics were classified into 8 different classes according to their chemical structure: penicillins (PN; amoxicillin, ampicillin), cephalosporines (CPS; ceftazidime, cephalothin, cefuroxime), guinolones (Q; nalidic acid), aminoglycosides (AMG; amikacin, doxycyclin, erythromycin, streptomycin, tobramycin, gentamicin, kanamycin, neomycin), tetracyclines (TET; tetracycline), sulfonamides (SUF; trimethoprin), rifampicins (RIF; rifampicin), other (O; chloramphenicol, nitrofurantoin, novobiocin). The basic antibiotic resistance mechanisms were not considered.

The test to evaluate the biocides resistance was performed accordingly. 10 μl of the biocides was added to the sterilized disks (Oxoid). The following commercial biocides were used: Preventol® (LANXESS; GDA50, D2, BIT20N) and Acticide® (THOR; LV508, OX, MBF). Both type of the biocides provide safe and effective protection against the microbiological spoilage of a wide variety of products, including paints, polymer emulsions, adhesives, inks, metalworking fluids, detergents, pulp/paper and water treatment, wood, plastics and leather.

All the experiments were carried out in 3 replicates.

Evaluation of Cell Surface Hydrophobicity

The hydrophobicity of the isolates was evaluated by the two methods: BAH (Bacterial Adhesion to Hydrocarbon Test) and SAT (Salt Aggregation Test).

BAH Experiment was carried out using the procedure described by Rosenberg et al. (1980) Bacteria were harvested during the exponential growth phase by centrifugation at 9000 x g for 20 min. After centrifugation, cells were washed twice with PBS (pH = 7), and resuspended in the same buffer to fit an optical density of ca. 0.8 (A_o). Optical density was measured at 600 nm on a UV-Visible Spectrophotometer Shimadzu. Next, 500 μl of hexadecane was added to 5 ml of bacterial suspension and vortexed for 2 min, and allowed to stand until the phase separated. Then the aqueous-phase was carefully removed and its absorbance was measured (A_1).

The hydrophobicity was calculated using the formula:

$$\text{Adhesion \%} = (A_o - A_1)/A_o \times 100$$

Isolates with BAH greater than 70% are classified as highly hydrophobic; isolates with BAH between 50 and 70% are classified as moderate, and isolates with BAH lower than 50% but great than 10% are classified as low hydrophobic.

SAT Experiment was carried out using the procedure described by Lindhal et al. (1981). 50 μl of bacterial suspensions in PBS (1.5 x 10^8 CFU/ml) were mixed with 50 μl of different concentrations (from 3.0 M to 0.1 M) of $(NH_4)_2SO_4$. The bacteria/salt solution mixture was gently rocked for 2 min, and cell aggregation was visual observed in each concentration. Isolates were divided into 4 groups: bacteria with strong hydrophobicity (cell autoaggregation in 3% NaCl); bacteria with medium hydrophobicity (aggregation between 0.1 – 1.0 M $(NH_4)_2SO_4$); bacteria with low hydrophobicity (aggregation between 1.2 -1.5 M $(NH_4)_2SO_4$); hydrophilic bacteria (aggregation ≥ 2 M $(NH_4)_2SO_4$).

All the procedures was repeated 3 times.

Results and Discussion

The biochemical characterization *E. coli* isolates obtained from the wastewater effluent of Strzegowa WWTP is presented in Table 1.

The resistance and sensitivity frequency to 20 tested antibiotics for all isolates is presented in Fig. 1.

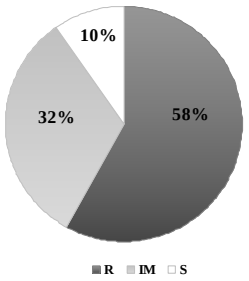


FIG. 1 THE PROFILE OF *E. COLI* ISOLATES TO 20 TESTED ANTIBIOTICS R – RESISTANCE, IM – INTERMEDIATE, S - SENSITIVITY

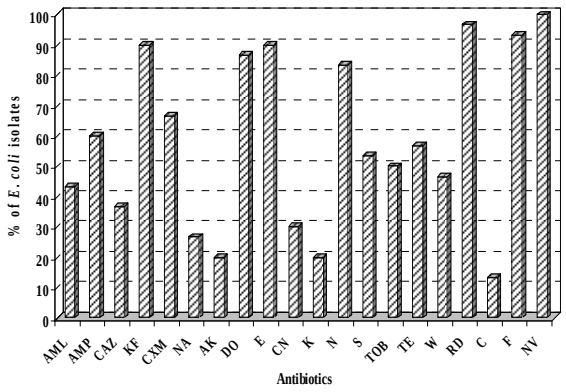
TABLE 1BIOCHEMICAL CHARACTERIZATION OF E. COLI ISOLATES

Enzymes	*E. coli* isolates																													
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	E22	E23	E24	E25	E26	E27	E28	E29	E30
control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaline phosphatase	+++	++	+++	-	+++	+++	+++	+++	+++	+++	+	+	++	-	+++	+++	+++	+++	+	+++	+++	+++	+++	+	+	+	+	+++	+	+
Esterase (C4)	+	+	++	+	++	+	++	++	+	++	+	+	+	++	+	+	++	+	+	+	+	++	-	+	+	+	+	+	+	-
Esterase Lipase (C8)	-	-	+	-	-	-	-	-	-	-	-	-	-	++	+	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-
Lipase (C14)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
Leucine arylamidase	+++	+	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	+++	+++	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
Valine arylamidase	+	-	+	-	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-
Cystine arylamidase	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	-	-	+	-	-	+	-	-
Trypsin	-	-	-	-	+	+	+	++	++	-	+	++	+	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-
α-chymotrypsin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Acid phosphatase	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	++
Naphthol-AS-BI-phosphohydroiase	+++	+++	+++	++	+++	++	++	+++	+	+++	+++	+++	+++	+	++	+++	+++	+	+++	+++	+++	++	++	+	++	+++	+++	+++	+++	++
α-galactosidase	-	-	-	+	-	+	-	-	-	+	-	-	-	-	+	+	-	+	+	-	-	-	-	+	-	+	+	++	+	+
β-galactosidase	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	++	++	+++	++	+++	++	++	++	++	++	++	+++	++	+++	+++	+++
β-glucuronidase	+	+	+	-	+++	++	+	+	+++	+++	+	-	+	-	-	+	+	+	+	+	+	+	-	-	-	+	+	++	-	+
α-glucosidase	+	++	++	+	+++	++	++	+++	+++	++	++	+	+	-	++	++	+	+	+++	+	+	+	+	+	-	++	+	++	+	++
β-glucosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N-acetyl-β-glucosaminidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-mannosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-fucosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+++ high activity; ++ modern activity; + low activity; - no activity

The data indicates that antibiotic resistant bacteria represent big part of the total isolates (58%). Mudryk et al. (2010) classify the strains which show resistance or intermediate behavior as "resistant". All others strains were classified as sensitive. According to this classification, 90% of *E. coli* isolated were categorized as resistant. The highest resistance frequency: 90%, 94%, 97% and 100% of studied strains was related to cephalotin and erythromycin, nitrofuzantoin, rifampicin and novobiocin, respectively (Fig. 2A and 2B).

A.



B.

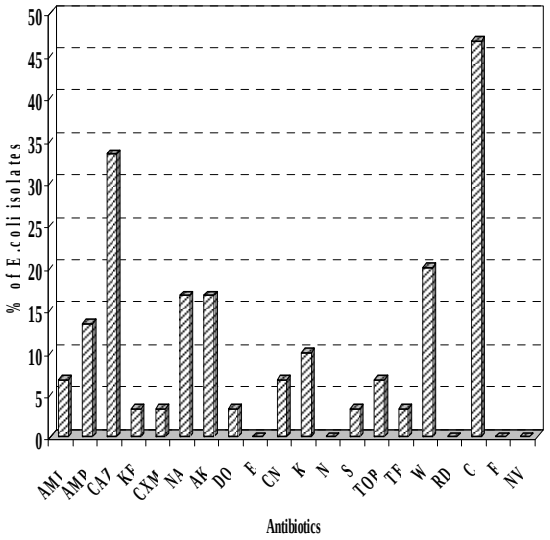


FIG. 2 RESISTANCE % (A) AND SENSITIVITY % (B) TO 20 ANTIBIOTICS AMONG *E. coli* ISOLATES

The collection of bacterial strains was also analyzed for multiple antibiotic resistance (Fig. 3). The data indicated that 88% of bacteria showed resistance to 8 - 15 antibiotics. 3% of isolates were resistance to 19 tested antibiotics. No strain was detected to be resistance to 0 – 5 antibiotics.

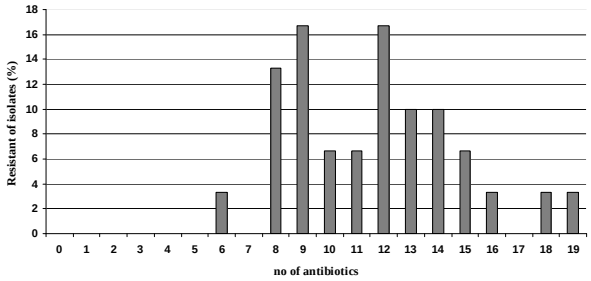
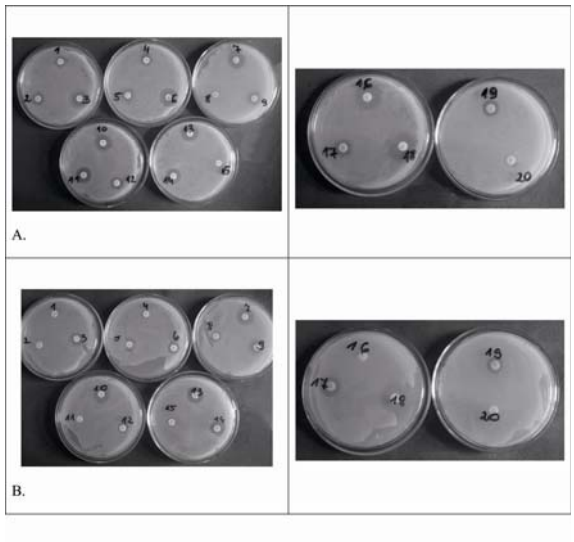


FIG. 3 INCIDENCE OF MULTIPLE ANTIBIOTIC RESISTANCE (MAR) AMONG *E. coli* STRAINS



FOT. 1 ANTIBIOTICS RESISTANT OF E 12(A) AND E 17(B) ISOLATES

ANTIBIOTICS: 1-AMOXYCILLIN; 2-AMPICILLIN; 3-CEFTAZIDIME; 4- CEPHALOTHIN; 5-CEFUROXIME; 6-NALIDIC ACID; 7-AMIKACIN; 8-DOXYCYCLIN; 9-ERYTHROMYCIN; 10-GENTAMICIN; 11-KANAMYCIN; 12-NEOMYCIN; 13-STREPTOMYCIN; 14-TOBRAMYCIN; 15-TETRACYCLINE; 16-TRIMETHOPRIM; 17-RIFAMPICIN; 18-CHLORAMPHENICOL; 19-NITROFURANTOIN; 20-NOVOBIOCIN

The wide application of antibiotics in human and veterinary medicine has led to large scale dissemination of bacteria resistant to antibiotics in different part of the environment. The main sources of resistant bacteria are manure and liquid manure of animals as well as human excretions. They serve as a reservoir for bacteria with multiple resistance. Endogenous bacterial flora plays an important role as acceptor and donor of transmissible drug resistance genes. *Escherichia coli* is commonly detected in the intestinal tract of humans and animals, and can also be implicated in human and animal infectious diseases. There are some papers in which antibiotic susceptibility of *E. coli* isolates from healthy humans or animals and foods has been studied (Saenz et al. 2001; Schroeder et al. 2002; Klein et al. 2003; Srinivasan et al. 2007). However, few studies have focused on the antibiotic resistance bacteria, among them E. *coli*, in the aquatic environment (Schwartz et al. 2003; Reinthaler et al. 2003; Korzeniewska et al. 2013). Reinthaler et al. (2003) evaluated resistance patterns of *E. coli* in wastewater treatment plants (WWTPs). Investigations have been done in sewage, sludge and receiving waters from the WWTPs. The highest resistance rates found in isolated *E. coli* strains were the following groups: penicillin (ampicillin and piperacillin), cephalosporin group (cefalothin and cefuroxime), quinolones (nalidixic acid) and for trimethoprime/sulfamethoxazole, and for tetracycline. Of all the antimicrobial substances investigated, the highest rate of resistance was noted for tetracycline (up to 57%). Korzeniewska et al. (2013) investigated the contamination degree of hospital effluents and municipal sewage (inflow, sewage in aeration tank, outflow) with antibiotic-resistant and beta-lactamases producing *E.coli* strains. As well, *E. coli* strains emission to the air near selected WWTP facilities and to the river, where the treated effluent was discharged, was determined. The results obtained by the authors indicated that antibiotic-resistant *E.coli* strains were emitted from sewage to the atmospheric air near WWTPs and their surroundings or directly into the water sources. Further investigations are required concerning affect of antibiotic resistance strains released from WWTPs on the surface waters, soil and air as well as future evaluation and control needed to evaluate and reduce public health risk.

The effect of biocides in *E. coli* isolates is presented in Table 2.

TABLE 2EFFECT OF BIOCIDES ON *E. COLI* ISOLATES

Isolates	INHIBITION ZONE (mm)					
	PREVENTOL®			ACTICIDE®		
	GDA50	D2	BIT20N	LV 508	OX	MBF
E1	27.50 ± 2.12	45.00 ± 0.00	34.00 ± 5.66	55.50 ± 2.12	43.00 ± 1.41	26.00 ± 4.24
E2	23.50 ± 0.71	44.00 ± 5.66	26.50 ± 0.71	48.00 ± 4.24	44.50 ± 2.12	21.00 ± 0.00
E3	25.50 ± 2.12	50.50 ± 0.71	40.00 ± 2.83	47.00 ± 1.41	34.50 ± 0.71	19.50 ± 2.12
E4	24.50 ± 3.54	60.50 ± 0.71	29.50 ± 2.12	54.00 ± 2.83	43.00 ± 4.24	24.00 ± 2.83
E5	28.00 ± 1.41	60.00 ± 0.00	37.00 ± 2.83	47.00 ± 0.00	46.00 ± 2.83	24.00 ± 1.41
E6	24.50 ± 4.95	50.00 ± 14.14	30.50 ± 6.36	51.50 ± 2.12	43.00 ± 2.83	24.50 ± 2.12
E7	24.08 ± 1.41	53.50 ± 0.71	28.50 ± 2.12	49.00 ± 1.41	44.00 ± 2.83	21.50 ±1.41
E8	24.00 ± 7.07	53.00 ± 2.83	29.50 ± 2.12	46.00 ± 2.83	46.00 ± 4.24	25.00 ± 2.83
E9	23.50 ± 2.12	60.50 ± 0.71	33.50 ± 0.71	49.00 ± 1.41	47.00 ± 4.24	26.50 ± 2.12
E10	28.00 ± 2.83	60.00 ± 0.00	31.50 ± 3.54	48.50 ± 0.71	44.00 ± 1.41	22.50 ± 0.71
E11	25.50 ± 2.12	53.00 ± 9.90	22.50 ± 0.71	42.50 ± 3.54	42.50 ± 0.71	23.50 ± 0.71
E12	24.00 ± 1.41	52.50 ± 3.54	27.00 ± 2.83	49.50 ± 6.36	39.00 ± 1.41	21.50 ± 0.71
E13	23.50 ± 2.12	52.50 ± 0.71	29.00 ± 2.83	44.50 ± 0.71	50.00 ± 5.66	20.50 ± 4.95
E14	32.50 ± 0.71	48.00 ± 4.24	30.50 ± 3.54	47.50 ± 2.12	40.00 ± 7.07	23.00 ± 2.83
E15	26.00 ± 5.66	46.50 ± 3.54	31.00 ± 2.83	45.50 ± 0.71	37.50 ± 2.12	18.00 ± 1.41
E16	28.00 ± 1.41	49.00 ± 1.41	29.50 ± 0.71	49.00 ± 2.83	40.00 ± 2.83	20.00 ± 0.00
E17	29.50 ± 0.71	49.50 ± 0.71	30.50 ± 2.12	48.50 ± 2.12	39.00 ± 1.41	22.50 ± 0.71
E18	29.50 ± 3.54	47.50 ± 3.54	25.50 ± 0.71	50.00 ± 1.41	39.00 ± 5.66	19.50 ± 2.12
E19	28.50 ± 2.12	50.50 ± 0.71	28.50 ± 3.54	54.50 ± 0.71	46.00 ± 1.41	26.00 ± 1.41
E20	26.50 ± 3.54	47.00 ± 2.83	33.50 ± 0.71	46.00 ± 1.41	37.50 ± 2.12	18.50 ± 0.71
E21	29.00 ± 2.83	48.50 ± 3.54	31.00 ± 4.24	53.00 ± 0.00	48.00 ± 2.83	25.50 ± 2.12
E22	28.00 ± 2.83	47.00 ± 0.00	30.00 ± 0.00	51.50 ± 2.12	48.00 ± 1.41	24.00 ± 1.41
E23	23.50 ± 2.12	37.50 ± 2.12	34.50 ± 1.26	47.50 ± 0.71	39.50 ± 3.54	19.50 ± 0.71
E24	28.50 ± 3.54	48.00 ± 1.41	29.00 ± 0.00	48.00 ± 1.41	43.50 ± 2.12	24.50 ± 2.12
E25	26.50 ± 0.71	46.50 ± 3.54	28.50 ± 0.71	48.00 ± 2.83	39.50 ± 3.54	22.50 ± 2.12
E26	28.50 ± 6.36	46.50 ± 0.71	26.00 ± 5.66	45.50 ± 2.12	45.00 ± 0.00	22.50 ± 2.12
E27	29.00 ± 1.41	49.00 ± 2.83	28.50 ± 0.71	46.00 ± 1.41	38.50 ± 0.71	20.00 ± 1.41
E28	25.50 ± 2.12	46.50 ± 2.12	26.50 ± 4.95	53.00 ± 1.41	44.50 ± 3.54	27.00 ± 1.41
E29	31.50 ± 3.54	57.50 ± 3.54	36.00 ± 2.83	51.50 ± 3.54	43.50 ± 3.54	21.50 ± 0.71
E30	25.00 ± 1.41	28.50 ± 3.54	26.50 ± 0.71	53.00 ± 2.83	38.00 ± 2.83	20.50 ± 0.71

Mean ± Stand. Dev.

D2 biocide was the most active among the Preventol® group. The similar activity showed LV508 biocide from the Acticide® group. Generally, the bacteria was susceptibility to all tested biocides. Biocides are used to reduce or eliminate both pathogens and spoilage microorganisms. However, bacteria have been shown to adopt to environmental conditions and stresses designed to counteract them. An increase in biocide tolerance in an important public health issue. Sheidan et al. (2012) evaluated the level of tolerance among *E. coli* and its serotypes to commonly used biocides in the food processing. In the study, most of the commercial biocide tested were effective. However, their findings have also indicated that misuse and/or overuse of the biocides could affect biocide tolerance among foodborne pathogens.

The review written by Russell (2003) is comprehensive knowledge on biocide and antibiotic actions, their similarities and differences. This article has explored some important aspect of antibiotic and biocide activities, for example: whether biocide use could lead to the development or induction of a mechanism that results in new or increased antibiotic resistance and the findings from laboratory tests, in which many stresses are controlled, relevant to the clinical and environmental situations where the stresses are uncontrolled.

In this study, *E. coli* strains were resistance to different antibiotics and simultaneously they were susceptibility to all tested biocides. One possible explanation for the rapid development of resistance is based on the mutator hypothesis, which may account for a speeded up evolutionary process in *E. coli*. LeClerc et al. (1996) demonstrated that more than 1% of *E. coli* O157 strains had spontaneous rates of mutation 1000-times higher than other strains. Braoudaki and Hilton (2004) presented data on cross-resistance of triclosan-adapted different *E. coli* serotypes to a range of antimicrobial agents and demonstrated decrease susceptibility of tested strains to a wide panel of antimicrobial agents including chloramphenicol, tetracycline, amoxicillin, amoxicillin/clavulanic acid and trimethoprim as well as to biocides benzalkoniumchloride and chlorhexidine. Antibiotic resistance of *E. coli* strains may arise by mutation or adaptation or by the acquisition of plasmids, transposons or other genetic elements. Mutational resistance to antibiotics is a well-known mechanism (Russell 2003). However, target-site mutations are rare with biocides. The decrease of susceptibility of bacterial isolates to antibiotics was probably caused by the presence of the compounds in the wastewaters and the long exposition of *E. coli* strains to them. The evolutionary nature of antibiotic resistance and its possible relation to biocide use needs to be establish (Russell 2003).

Different mechanisms to be ineffective for the antibiotics used against bacteria (Schwartz et al. 2003) have been developed. The genes encoding the antibiotic-resistance mechanisms are located on the bacterial chromosomes or on extrachromosomal plasmids, and transmitted to the next generation–vertical gene transfer or can also be exchanged among bacteria of different taxonomic position–horizontal gene transfer.

In Figure 4, hydrophobicity of *E. coli* strains evaluated by the BAH method is presented. Only 17% of the isolates revealed moderate hydrophobicity. The rest of bacteria showed no and low hydrophobicity. Most of the bacteria was hydrophilic.

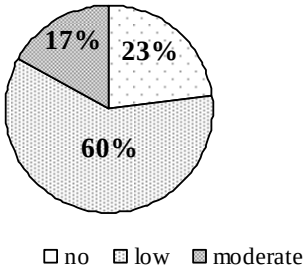


FIG. 4 HYDROPHOBICITY OF *E. COLI* ISOLATES EVALUATED BY BAH METHOD

The similar results were obtained in the SAT method. 80% of *E. coli* aggregated in 1.2 – 1.5 M $(NH_4)_2SO_4$. Lindahl et al. (1981) found that eneropathogenic *E. coli* strains were more hydrophobic than the nonpathogenic variants, and the observation could make the basis for a new method for treatment of infections. Cell–cell adhesion is of fundamental importance in different biological systems. The mechanisms by which cells can bind/communicate to each other are numerous and varied including electrostatic and hydrophobic interactions, hydrogen bonds, and also different substances are involved in the signalling processes (Galloway et al. 2012). Galloway et al. (2012) in their review described some quorum sensing-regulated mechanisms that have significant impacts upon agriculture, environment and human healthcare.

In summary, despite significant progress in recent years, the field of antimicrobial resistance patterns of *E. coli* serotypes, in may regards, has been still in its

infancy. The wastewater treatment plant constitutes an important reservoir of bacteria which carry potentially transferable resistance genes. They can move to the soils, surface waters, ground waters, drinking waters and also to the air as bioaerosols. Inhalation of bioaerosols can cause a variety of lung illnesses. In the environmental risk assessment, the importance of resistant bacteria and genes migrations should be taken into consideration. Development in both the practical and theoretical aspects of antimicrobial resistance is needed. Considerable progress will be witnessed in the forthcoming year in the significance of environmental contamination posses by antibiotic resistant genes and bacteria for human health.

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